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Signed Andrew Gerry

Dated 2 June 2003

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1/77

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1. Your reference — KC/RSG/B45322

2. Patent application number
(The Patent Office will fill in his part) — 0221689.3 — 18 SEP 2002

3. Full name, address and postcode of the or of each applicant — GlaxoSmithKline Biologicals s.a.
Rue de l'Institut 89, B-1330 Rixensart, , Belgium

Patents ADP number *(if you know it)* 20473587003

If the applicant is a corporate body, give the country/state of its incorporation — Belgian

see continuation sheet for further applicant(s)

4. Title of the invention — Vaccine

5. Name of your agent *(if you have one)* — Corporate Intellectual Property

"Address for service" in the United Kingdom to which all correspondence should be sent
(including the postcode)

GlaxoSmithKline
Corporate Intellectual Property (CN9 25.1)
980 Great West Road
BRENTFORD
Middlesex TW8 9GS

Patents ADP number *(if you know it)* 80725550006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of

this request? *(Answer yes if:*

a) any applicant named in part 3 is not an inventor, or

b) there is an inventor who is named as an applicant, or

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Patents Form 1/77

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Continuation sheets of this form	0
Description	35
Claim(s)	3
Abstract	1
Drawings	11

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Priority Documents

Translations of priority documents

Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*

Request for preliminary examination and search *(Patents Form 9/77)*

Request for substantive examination *(Patents Form 10/77)*

Any other documents *(please specify)*

11. We request the grant of a patent on the basis of this application

Signature R J Stephen Date 18-Sep-02

12. Name and daytime telephone number of person to contact in the United Kingdom

R J Stephen 020 80474466

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Patents Form 1/77

Vaccine

The present invention relates to pharmaceutical compositions and methods for inducing an immune response against tumours-related antigens. More specifically, the invention relates to non-human prostate-specific antigens which can be used as xenogeneic antigens to induce prostate-directed immunity in humans, to pharmaceutical compositions containing them, to methods of manufacture of such compositions and to their use in medicine.

In particular the compositions of the invention include the prostate-specific protein known as P501S, from a non human origin. Such compositions find utility in cancer vaccine therapy, particularly prostate cancer vaccine therapy and diagnostic agents for prostate tumours. The present invention also provides methods for formulating vaccines for immunotherapeutically treating prostate cancer patients and P501S-expressing tumours other than prostate tumours, prostatic hyperplasia, and prostate intraepithelilial neoplasia (PIN).

Prostate cancer is the most common cancer among males, with an estimated incidence of 30% in men over the age of 50. Overwhelming clinical evidence shows that human prostate cancer has the propency to metastasise to bone, and the disease appears to progress inevitably from androgen dependent to androgen refractory status, leading to increased patient mortality (Abbas F., Scardino P. "The Natural History of Clinical Prostate Carcinoma." *In* Cancer (1997); 80:827-833). This prevalent disease is currently the second leading cause of cancer death among men in the US.

Despite considerable research into therapies for the disease, prostate cancer remains difficult to treat. Currently, treatment is based on surgery and/or radiation therapy, but these methods are ineffective in a significant percentage of cases (Frydenberg M., Stricker P., Kaye K. "Prostate Cancer Diagnosis and Management "The Lancet (1997); 349:1681-1687). Several tumour-associated antigens are already known. Many of these antigens may be interesting targets for immunotherapy, but are either not fully tumour-specific or are closely related to normal proteins, and hence bear with them the risk of organ-specific auto-immunity, once targeted by a potent immune response. When an auto-immune response to non-crucial organs can be tolerated, auto-immunity to heart, intestine and other crucial organs could lead to unacceptable safety profiles. Some previously identified prostate specific proteins like

prostate specific antigen (PSA) and prostatic acid phosphatase (PAP), prostate-specific membrane antigen (PSMA) and prostate stem cell antigen (PSCA) used in vaccine preparations have only showed limited therapeutic potential so far (Pound C., Partin A., Eisenberg M. et al. "Natural History of Progression after PSA Elevation following Radical Prostatectomy." *In* Jama (1999); 281:1591-1597) (Bostwick D., Pacelli A., Blute M. et al. "Prostate Specific Membrane Antigen Expression in Prostatic Intraepithelial Neoplasia and Adenocarcinoma." *In* Cancer (1998); 82:2256-2261), and this limitation may be due to a relatively poor immunogenicity due to their self nature, or by poor prostate and tumour-specificity.

The existence of tumour rejection mechanisms has been recognised since several decades. Tumour antigens, though encoded by the genome of the organism and thus theoretically not recognized by the immune system through the immune tolerance phenomenon, can occasionally induce immune responses detectable in cancer patients. This is evidenced by antibodies or T cell responses to antigens expressed by the tumour (Xue BH., Zhang Y., Sosman J. et al. "Induction of Human Cytotoxic T-Lymphocytes Specific for Prostate-Specific Antigen." *In* Prostate (1997); 30(2):73-78). When relatively weak anti-tumour effects can be observed through the administration of antibodies recognizing cell surface markers of tumour cells, induction of strong T cell responses to antigens expressed by tumour cells can lead to complete regression of established tumours in animal models (mainly murine).

It is now recognised that the expression of tumour antigens by a cell is not sufficient for *induction* of an immune response to these antigens. Initiation of a tumour rejection response requires a series of immune amplification phenomena dependent on the intervention of antigen presenting cells, responsible for delivery of a series of activation signals.

Human P501S as described in WO 98/37418, and its C-terminal fragments PS108 as described in WO 98/50567 and Y54369 as described in WO 99/67384, is a human prostate specific antigen, associated with a prostate tissue disease or condition, especially with prostate cancer. Its expression is observed in normal and tumour prostate tissue as well as in some breast metastasis (WO 00/61756).

P501S is a membrane protein which interacts with a cell surface receptor. It is predicted to be a type IIIa plasma membrane protein with 9-11 transmembrane

regions spanning the whole length of the protein. P501S shares some homologies with spinash sucrose binding protein (Riesmeier JW, Willmitzer L, Frommer WB, 1992, EMBO J 11, 4705-13).

P501S nucleotide sequence and deduced polypeptide sequence and fragments are disclosed in WO 98/37418. Contiguous and partially overlapping cDNA fragments and polypeptides encoded thereby, have also been described (WO 98/50567), more particularly a C-terminal fragment of 255 amino acids in length. A polypeptide of 231 amino acids in length, described in WO 99/67384, is reported to comprise a potential transmembrane domain, two potential caseine kinase II phosphorylation sites, one potential protein kinase C phosphorylation site and a potential cell attachment sequence.

P501S is described as being a member of the family of human "self" antigens", against which it will be suposedly difficult to induce an "auto-immune" response, including CD8+ cytotoxic T-lymphocyte (CTL) responses. Therefore efficient vaccine strategies directed against P501S will require the development of methods to overcome the immune tolerance to the self-protein.

The present invention is concerned with an efficient antigen-specific immunotherapy of human malignancies, more especially of human prostate cancer. It takes advantage of the surprising observation that humans immunised with an antigen from a xenogeneic (non human) origin, are capable of mounting a effective immune response against the human antigen counterpart, through the generation of cross-reactive antibodies and/or T cells. Such an approach has the advantages over classical immunotherapy that utilises human prostate self antigens, since these antigens are tolerated by the human body and it is therefore difficult to raise an immune response against the antigen.

Accordingly, the present invention provides methods for purifying the xenogeneic P501S antigens and for formulating vaccines for immunotherapeutically treating P501S-expressing prostate tumors, prostatic hyperplasia and prostate intraepithelilial neoplasia (PIN).

The present invention also provides pharmaceutical compositions and vaccine compositions suitable for use in medicine, and more especially in the treatment of a prostate tumours, said composition comprising a xenogeneic P501S antigen. More particularly, the invention is directed to a mouse, rat and monkey P501S which can be

used as a xenogeneic form of human P501S antigen to induce prostate-targeted immunity in humans.

A xenogeneic form of antigen refers to an antigen having substantial sequence identity to the human antigen (also termed autologous antigen) which serves as a reference antigen but which is derived from a different non-human species. In this context the substantial identity refers to concordance of an amino acid sequence with another amino acid sequence or of a polynucleotide sequence with another polynucleotide sequence when such sequence are arranged in a best fit alignment in any of a number of sequence alignment proteins known in the art. By substantial identity is meant at least 70-98%, and preferably at least 85-95% sequence identity between the compared sequences. Therefore according to the invention the xenogeneic P501S will be a P501S polypeptide which is xenogeneic with respect to human P501S, in other words which is isolated from a species other than human. In a preferred embodiment, the polypeptide is isolated from mouse, rat, or Cynomolgus monkey (Maccaca fascicularis). In a more preferred embodiment, the P501S polypeptide has the sequence set forth in SEQ ID NO:1 (rat), in SEQ ID NO:3 (Cynomolgus monkey) or in SEQ ID NO:10 (mouse). The isolated xenogeneic P501S polypeptide will generally share substantial sequence similarity, and include isolated polypeptides comprising an amino acid sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:10 over the entire length of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:10 respectively. Accordingly the polypeptide will comprise an immunogenic fragment of the polypeptide SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:10 in which the immunogenic activity of the immunogenic fragment is substantially the same as the polypeptide SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:10 respectively. The polypeptide sequence as set forth in SEQ ID NO:1 and the polynucleotide sequence as set forth in SEQ ID NO:2 are novel and also form part of the invention. In particular the invention provides an isolated polypeptide comprising an amino acid sequence which has at least 90%, preferably at least 92% identity to the amino acid sequence of SEQ ID NO:1 over the entire length of of SEQ ID NO:1. Preferably the isolated polypeptide amino acid sequence has at least 95% identity to SEQ ID NO:1. Still more preferably the polypeptide comprises the amino

acid sequence of SEQ ID NO:1. Most preferably the polypeptide is the isolated polypeptide of SEQ ID NO:1.

In addition the polypeptide can be a fragment of at least about 20 consecutive amino acids, preferably about 30, more preferably about 50, yet more preferably about 100, most preferably about 150 contiguous amino acids selected from the amino acid sequences as shown in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:10. More particularly fragments will retain some functional property, preferably an immunological activity, of the larger molecule set forth in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:10, and are useful in the methods described herein (e.g. in pharmaceutical and vaccine compositions, in diagnostics, etc.). In particular the fragments will be able to generate an immune response against the human counterpart, such as the generation of cross-reactive antibodies which react with the autologous human form of P501S as set forth in any of the SEQ ID NO: 5 (Corixa WO 98/37418), SEQ ID NO: 6 (Abbott WO 98/50567) and SEQ ID NO:7 (Incyte WO 99/67384).

In one embodiment, the polypeptide of the invention may be part of a larger fusion, comprising the tumour-associated xenogeneic P501S or fragment thereof and a heterologous protein or part of a protein acting as a fusion partner. The protein and the fusion partner may be chemically conjugated, but are preferably expressed as recombinant fusion proteins in a heterologous expression system. In a preferred embodiment of the invention there is provided a xenogeneic P501S fusion protein linked to an immunological fusion partner that may provides additional T helper epitopes thereby further assisting in breaking the tolerance against the autologous antigen. Thus the fusion partner may act through a bystander helper effect linked to secretion of activation signals by a large number of T cells specific to the foreign protein or peptide, thereby enhancing the induction of immunity to the P501S component as compared to the non-fused xenogeneic protein. Preferably the heterologous partner is selected to be recognizable by T cells in a majority of humans.

In another embodiment, the invention provides a xenogeneic P501S protein or fragment or homologues thereof linked to a fusion partner that acts as an expression enhancer. Thus the fusion partner may assist in aiding in the expression of P501S in a heterologous system, allowing increased levels to be produced in an expression system as compared to the native recombinant protein.

Preferably the fusion partner will be both an immunological fusion partner and an expression enhancer partner thereby assisting in aiding the expressing and in breaking the tolerance against the autologous antigen. Accordingly, the present invention in the embodiment provides fusion proteins comprising the tumour-specific P501S or a fragment thereof linked to a fusion partner. Preferably the fusion partner is acting both as an immunological fusion partner and as an expression enhancer partner. Accordingly, in a preferred form of the invention, the fusion partner is the non-structural protein from influenzae virus, NS1 (hemagglutinin) or fragment thereof. Typically the N-terminal 81 amino acids are utilised, although different fragments may be used provided they include T-helper epitopes (C. Hackett, D. Horowitz, M. Wysocka & S. Dillon, 1992, J. Gen. Virology, 73, 1339-1343). When NS1 is the immunological fusion partner it has the additional advantage in that it allows higher expression yields to be achieved. In particular, such fusions are expressed at higher yields than the native recombinant P501S proteins. In another preferred form of the invention, the immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium, Haemophilus influenza B (WO91/18926). Preferably the protein D derivative comprises approximately the first 1/3 of the protein, in particular approximately the first N-terminal 100-110 amino acids. Preferably the protein D derivative is lipidated. Preferably the first 109 residues of the Lipoprotein D fusion partner is included on the N-terminus to provide the vaccine candidate antigen with additional exogenous T-cell epitopes and increase expression level in *E-coli* (thus acting also as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. In another embodiment the immunological fusion partner is the protein known as LYTA. Preferably the C terminal portion of the molecule is used. Lyta is derived from Streptococcus pneumoniae which synthesize an N-acetyl-L-alanine amidase, amidase LYTA, (coded by the lytA gene {Gene, 43 (1986) page 265-272} an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E.coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. As used herein a

preferred embodiment utilises the repeat portion of the Lyta molecule found in the C terminal end starting at residue 178. A particularly preferred form incorporates residues 188 - 305. In another preferred embodiment, the immunological fusion partner is derived from a Mycobacterium sp., such as a Mycobacterium tuberculosis-derived Ra12 fragment. Ra12 refers to a polynucleotide region that is a subsequence of a *Mycobacterium tuberculosis* MTB32A nucleic acid. MTB32A is a serine protease of 32 KD molecular weight encoded by a gene in virulent and avirulent strains of *M. tuberculosis*. The nucleotide sequence and amino acid sequence of MTB32A have been described (for example Skeiky *et al., Infection and Immun.* (1999) 67:3998-4007). C-terminal fragments of the MTB32A coding sequence express at high levels and remain as a soluble polypeptides throughout the purification process. Moreover, Ra12 may enhance the immunogenicity of heterologous immunogenic polypeptides with which it is fused. One preferred Ra12 fusion polypeptide comprises a 14 KD C-terminal fragment corresponding to amino acid residues 192 to 323 of MTB32A. Other preferred Ra12 polynucleotides generally comprise at least about 15 consecutive nucleotides, at least about 30 nucleotides, at least about 60 nucleotides, at least about 100 nucleotides, at least about 200 nucleotides, or at least about 300 nucleotides that encode a portion of a Ra12 polypeptide. Ra12 polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a Ra12 polypeptide or a portion thereof) or may comprise a variant of such a sequence. Ra12 polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not substantially diminished, relative to a fusion polypeptide comprising a native Ra12 polypeptide.

The proteins of the present invention are expressed in an appropriate host cell, and preferably in *E. coli* or in yeast such as in *Pichia pastoris* or *Saccharomyces cerevisiae*. In a preferred embodiment the proteins are expressed with an affinity tag, such as for example, a histidine tail comprising between 5 to 9 and preferably six histidine residues, most preferably at least 4 histidine residues. These are advantageous in aiding purification through for example ion metal affinity chromatography (IMAC).

The present invention also provides a nucleic acid encoding the proteins of the present invention. In a preferred embodiment, the xenogeneic P501S polynucleotide

has the sequence set forth in SEQ ID NO:2 (rat) or in SEQ ID NO:4 (Cynomolgus monkey) or in SEQ ID NO:11 (mouse). The isolated xenogeneic P501S polynucleotides of the invention may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention. In other related embodiments, the present invention provides polynucleotide variants having substantial identity to the sequences disclosed herein in SEQ ID NO:2, in SEQ ID NO:4 or in SEQ ID NO:11, for example those comprising at least 70% sequence identity, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide sequence of this invention using the methods described herein, (*e.g.*, BLAST analysis using standard parameters). In a related embodiment, the isolated polynucleotide of the invention will comprise a nucleotide sequence encoding a polypeptide that has at least 90%, preferably 95% and above, identity to the amino acid sequence of SEQ ID NO:1, in SEQ ID NO:3 or in SEQ ID NO:10 over the entire length of SEQ ID NO:1, in SEQ ID NO:3 or in SEQ ID NO:10 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

Such sequences can be inserted into a suitable expression vector and used for DNA/RNA vaccination or expressed in a suitable host. The expression vectors comprising the isolated polynucleotide sequence according to the invention, and the appropriate hosts also form part of the invention. In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells *in vivo*. This may be achieved using any of a variety or well-known approaches. One of the preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of an expression vector such as a recombinant live viral or bacterial microorganism. Suitable viral expression vectors are for example poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelian Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), and herpesviruses (varicella zoster virus, etc). Other preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of a bacterial expression vector, such as Listeria, Salmonella , Shigella and BCG. Inoculation and in vivo infection with this live vector will lead to in vivo expression of the antigen and induction of immune responses.

These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

A DNA sequence encoding the proteins of the present invention can be synthesized using standard DNA synthesis techniques, such as by enzymatic ligation as described by D.M. Roberts *et al.* in Biochemistry 1985, *24*, 5090-5098, by chemical synthesis, by *in vitro* enzymatic polymerization, or by PCR technology utilising for example a heat stable polymerase, or by a combination of these techniques. Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10°-37°C, generally in a volume of 50µl or less. Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer, such as 0.05M Tris (pH 7.4), 0.01M $MgCl_2$, 0.01M dithiothreitol, 1mM spermidine, 1mM ATP and 0.1mg/ml bovine serum albumin, at a temperature of 4°C to ambient, generally in a volume of 50ml or less. The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982), or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, Nucleic Acids Research, 1982, 10, 6243; B.S. Sproat, and W. Bannwarth, Tetrahedron Letters, 1983, 24, 5771; M.D. Matteucci and M.H. Caruthers, Tetrahedron Letters, 1980, 21, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, 103, 3185; S.P. Adams *et al.*, Journal of the American Chemical Society, 1983, 105, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, Nucleic Acids Research, 1984, 12, 4539; and H.W.D. Matthes *et al.*, EMBO Journal, 1984, 3, 801.

In a further embodiment of the invention is provided a method of producing a protein as described herein. The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis *et al.*, Molecular Cloning - A Laboratory Manual; Cold Spring Harbor, 1982-1989. Accordingly there is provided a process for producing a xenogeneic polypeptide according to the invention, comprising culturing a host cell under conditions sufficient for the

production of said polypeptide and recovering the polypeptide from the culture medium. In particular, the process of the invention may preferably comprise the steps of:

i) preparing a replicable or integrating expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes the protein or an immunogenic derivative thereof;

ii) transforming a host cell with said vector;

ii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said protein; and

iv) recovering said protein.

The term 'transforming' is used herein to mean the introduction of foreign DNA into a host cell. This can be achieved for example by transformation, transfection or infection with an appropriate plasmid or viral vector using e.g. conventional techniques as described in Genetic Engineering; Eds. S.M. Kingsman and A.J. Kingsman; Blackwell Scientific Publications; Oxford, England, 1988. The term 'transformed' or 'transformant' will hereafter apply to the resulting host cell containing and expressing the foreign gene of interest. Preferably recombinant antigens of the invention are expressed in unicellular hosts, most preferably in bacterial systems, most preferably in *E. coli*.

The expression vectors are novel and also form part of the invention.

The replicable expression vectors may be prepared in accordance with the invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment encode the desired product, such as the DNA polymer encoding the protein of the invention, or derivative thereof, under ligating conditions.

Thus, the hybrid DNA may be pre-formed or formed during the construction of the vector, as desired.

The choice of vector will be determined in part by the host cell, which may be prokaryotic or eukaryotic but are preferably *E. coli*, yeast or CHO cells. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses. Expression and cloning vectors preferably contain a selectable marker such that only

the host cells expressing the marker will survive under selective conditions. Selection genes include but are not limited to the one encoding protein that confer a resistance to ampicillin, tetracyclin or kanamycin. Expression vectors also contain control sequences which are compatible with the designated host. For example, expression control sequences for *E. coli*, and more generally for prokaryotes, include promoters and ribosome binding sites. Promoter sequences may be naturally occurring, such as the β-lactamase (penicillinase) (Weissman 1981, *In Interferon 3* (ed. L. Gresser), lactose (lac) (Chang et al. Nature, 1977, 198: 1056) and tryptophan (trp) (Goeddel et al. Nucl. Acids Res. 1980, 8, 4057) and lambda-derived P_L promoter system. In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. This is the case for example for the tac synthetic hybrid promoter which is derived from sequences of the trp and lac promoters (De Boer et al., Proc. Natl Acad Sci. USA 1983, 80, 21-26). These systems are particularly suitable with *E. coli*.

Yeast compatible vectors also carry markers that allow the selection of successful transformants by conferring prototrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Control sequences for yeast vectors include promoters for glycolytic enzymes (Hess et al., J. Adv. Enzyme Reg. 1968, 7, 149), PHO5 gene encoding acid phosphatase, CUP1 gene, ARG3 gene, GAL genes promoters and synthetic promoter sequences. Other control elements useful in yeast expression are terminators and leader sequences. The leader sequence is particularly useful since it typically encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. Suitable signal sequences can be encoded by genes for secreted yeast proteins such as the yeast invertase gene and the a-factor gene, acid phosphatase, killer toxin, the a-mating factor gene and recently the heterologous inulinase signal sequence derived from INU1A gene of Kluyveromyces marxianus.. Suitable vectors have been developed for expression in *Pichia pastoris* and *Saccharomyces cerevisiae*.

A variety of *P. pastoris* expression vectors are available based on various inducible or constitutive promoters (Cereghino and Cregg, FEMS Microbiol. Rev. 2000,24:45-66). For the production of cytosolic and secreted proteins,the most commonly used P. pastoris vectors contain the very strong and tightly regulated alcohol oxidase (AOX1) promoter. The vectors also contain the *P. pastoris* histidinol dehydrogenase (HIS4) gene for selection in his4 hosts. Secretion of foreign protein

require the presence of a signal sequence and the *S. cerevisiae* prepro alpha mating factor leader sequence has been widly and successfully used in Pichia expression system. Expression vectors are integrated into the P. pastoris genome to maximize the stability of expression strains. As in *S.cerevisiae*, cleavage of a *P.pastoris* expression vector within a sequence shared by the host genome (AOX1 or HIS4) stimulates homologous recombination events that efficiently target integration of the vector to that genomic locus. In general, a recombinant strain that contains multiple integrated copies of an expression cassette can yield more heterologous protein than single-copy strain. The most effective way to obtain high copy number transformants requires the transformation of Pichia recipient strain by the sphaeroplast technique (Cregg et all 1985, Mol.Cell.Biol. 5: 3376-3385) .

The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis *et al.* cited above.

The recombinant host cell is prepared, in accordance with the invention, by transforming a host cell with a replicable expression vector of the invention under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Maniatis *et al.* cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

The choice of transforming conditions depends upon the choice of the host cell to be transformed. For example, in vivo transformation using a live viral vector as the transforming agent for the polynucleotides of the invention is described above. Bacterial transformation of a host such as *E. coli* may be done by direct uptake of the polynucleotides (which may be expression vectors containing the desired sequence) after the host has been treated with a solution of $CaCl_2$ (Cohen *et al.*, Proc. Nat. Acad. Sci., 1973, *69*, 2110) or with a solution comprising a mixture of rubidium chloride (RbC1), $MnCl_2$, potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbC1 and glycerol. Transformation of lower eukaryotic organisms such as yeast cells in culture by direct uptake may be carried out by using the method of Hinnen et al (Proc. Natl. Acad. Sci. 1978, 75 : 1929-1933). Mammalian cells in culture may be transformed using the calcium phosphate co-precipitation of the vector DNA onto the cells (Graham & Van der Eb, Virology 1978, 52, 546). Other methods for introduction of polynucleotides into mammalian cells include dextran

mediated transfection, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) into liposomes, and direct micro-injection of the polynucleotides into nuclei.

The invention also extends to a host cell transformed with a nucleic acid encoding the protein of the invention or a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Maniatis *et al.* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 50°C, preferably between 25°C and 35°C, most preferably at 30°C. The incubation time may vary from a few minutes to a few hours, according to the proportion of the polypeptide in the bacterial cell, as assessed by SDS-PAGE or Western blot.

The product may be recovered by conventional methods according to the host cell and according to the localisation of the expression product (intracellular or secreted into the culture medium or into the cell periplasm). Thus, where the host cell is bacterial, such as *E. coli* it may, for example, be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium or from cell free extracts. Where the host cell is a yeast such as *Saccharomyces cerevisiae* or *Pichia pastoris*, the product may generally be isolated from from lysed cells or from the culture medium, and then further purified using conventional techniques. The specificity of the expression system may be assessed by western blot using an antibody directed against the polypeptide of interest.

Conventional protein isolation techniques include selective precipitation, adsorption chromatography, and affinity chromatography including a monoclonal antibody affinity column. When the proteins of the present invention are expressed with a histidine tail (His tag), they can easily be purified by affinity chromatography using an ion metal affinity chromatography column (IMAC) column.

In a preferred embodiment of the invention the proteins of the present invention is provided with an affinity tag, such as a polyhistidine tail. In such cases the protein after the blocking step is preferably subjected to affinity chromatography. For those proteins with a polyhistidine tail, immobilised metal ion affinity

chromatography (IMAC) may be performed. The metal ion, may be any suitable ion for example zinc, nickel, iron, magnesium or copper, but is preferably zinc or nickel. Preferably the IMAC buffer contains detergent, preferably a non-ionic detergent such as Tween 80, or a zwitterionic detergent such as Empigen BB, as this may result in lower levels of endotoxin in the final product.

Further chromatographic steps include for example a Q-Sepharose step that may be operated either before of after the IMAC column. Preferably the pH is in the range of 7.5 to 10, more preferably from 7.5 to 9.5, optimally between 8 and 9, ideally 8.5.

The proteins of the present invention are provided either soluble in a liquid form or in a lyophilised form, which is the preferred form. It is generally expected that each human dose will comprise 1 to 1000 μg of protein, and preferably 30-300 μg.

The present invention also provides pharmaceutical and vaccine composition comprising xenogeneic P501S antigen or nucleic acid in a pharmaceutically acceptable excipient. Accordingly there is provided a process for the production of a pharmaceutical composition, comprising admixing a xenogeneic P501S polypeptide or a xenogeneic P501S-encoding polynucleotide with a suitable adjuvant, diluent or other pharmaceutically acceptable carrier.

More particularly the pharmaceutical and vaccine compositions of the invention comprise an effective amount of a xenogeneic P501S polypeptide or a xenogeneic P501S-encoding polynucleotide, and a pharmaceutically acceptable carrier. By effective amount is meant a dose of antigen that, when administered to a human, produces a detectable immune response, such as a humoral response (antibodies) or a cellular response. A preferred pharmaceutical composition comprises at least one xenogeneic P501S polypeptide having the sequence set forth in SEQ ID NO:1, in SEQ ID NO:3 or in SEQ ID NO:10 or an immunogenic fragment thereof. Said protein has, preferably, blocked thiol groups and is highly purified, e.g. has less than 5% host cell contamination. Another preferred pharmaceutical composition comprises at least one xenogeneic P501S-encoding polynucleotide having the sequence set forth in SEQ ID NO:2, in SEQ ID NO:4 or in SEQ ID NO:11 or a fragment thereof which encodes a polypeptide having retained some functional similarity with the protein of SEQ ID NO:1, in SEQ ID NO:3 or in SEQ ID NO:10.

Such vaccine may optionally contain one or more other tumour-associated antigen and derivatives from human or non-human origin. For example, suitable other associated antigen include PAP-1, PSA (prostate specific antigen), PSMA (prostate-specific membrane antigen), PSCA (Prostate Stem Cell Antigen), STEAP.

Vaccine preparation is generally described in Vaccine Design ("The subunit and adjuvant approach" (eds. Powell M.F. & Newman M.J). (1995) Plenum Press New York). Encapsulation within liposomes is described by Fullerton, US Patent 4,235,877.

The xenogenic proteins are preferably adjuvanted in the pharmaceutical or vaccine formulation of the invention. Suitable adjuvants are commercially available such as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); SBAS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

In the formulations of the invention it is preferred that the adjuvant composition induces an immune response predominantly of the TH1 type. High levels of Th1-type cytokines (*e.g.*, IFN-γ, TNFα, IL-2 and IL-12) tend to favour the induction of cell mediated immune responses to an administered antigen. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Accordingly, suitable adjuvants for use in eliciting a predominantly Th1-type response include, for example a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL) together with an aluminium salt. Other known adjuvants which preferentially induce a TH1 type immune response include CpG containing oligonucleotides. The oligonucleotides are characterised in that the CpG dinucleotide is unmethylated. Such oligonucleotides are well known

and are described in, for example WO 96/02555. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science 273*:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

A particularly potent adjuvant formulation involving QS21 3D-MPL & tocopherol in an oil in water emulsion is described in WO 95/17210 and is a preferred formulation.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), Detox (Ribi, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs).

Other preferred adjuvants include adjuvant molecules of the general formula (I):

$HO(CH_2CH_2O)_n$-A-R

Wherein, n is 1-50, A is a bond or –C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein *n* is between 1 and 50, preferably 4-24, most preferably 9; the *R* component is C_{1-50}, preferably C_4-C_{20} alkyl and most preferably C_{12} alkyl, and *A* is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-steoryl ether, polyoxyethylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the

Merck index (12th edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

Accordingly in one embodiment of the present invention there is provided a vaccine comprising a xenogeneic P501S of the present invention, which additionally comprises a TH-1 inducing adjuvant. A preferred embodiment is a vaccine in which the TH-1 inducing adjuvant is selected from the group of adjuvants comprising: 3D-MPL, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide. Another preferred embodiment is a vaccine comprising a xenogeneic P501S adjuvanted with a monophosphoryl lipid A or derivative thereof, QS21 and tocopherol in an oil in water emulsion.

Preferably the vaccine additionally comprises a saponin, more preferably QS21. Another particular suitable adjuvant formulation including CpG and a saponin is described in WO 00/09159 and is a preferred formulation. Most preferably the saponin in that particular formulation is QS21. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumour cells. Delivery vehicles include antigen-presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumour effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumour and peri-tumoural tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature 392*:245-251, 1998) and have been shown

to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumour immunity (*see* Timmerman and Levy, *Ann. Rev. Med. 50*:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel et al., *Nature Med. 4*:594-600, 1998). Accordingly there is preferably provided a vaccine comprising an effective amount of dendritic cells or antigen presenting cells, modified by in vitro loading with a polypeptide as described herein, or genetically modified in vitro to express a polypeptide as described herein and a pharmaceutically effective carrier.

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumour-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, lipopolysaccharide LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells. Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC,

adhesion molecules (*e.g.*, CD54 and CD11) and costimulatory molecules (*e.g.*, CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding P501S tumour protein (or derivative thereof) such that the P501S tumour polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., *Immunology and cell Biology 75*:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the P501S tumour polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (*e.g.*, vaccinia, fowlpox, adenovirus or lentivirus vectors).

Vaccines and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a vaccine or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

The present invention also provides a method of inducing an immune response against human P501S having an amino acid sequence as set forth in any of the sequences SEQ ID NO:5 to SEQ ID NO:7 in a human, comprising administering to the subject an effective dosage of a composition comprising a xenogeneic form of said human P501S as described herein. A preferred embodiment is a method of inducing an immune response against human P501S using the xenogeneic P501S isolated from mouse, rat or Cynomolgus monkey. Another preferred method of inducing an immune response according to the present invention is using an antigen composition including a live viral expression system which expresses said xenogeneic antigen said.

The present invention also provides a method for producing a vaccine formulation comprising mixing a protein of the present invention together with a pharmaceutically acceptable excipient, such as 3D-MPL.

Another aspect of the invention is the use of a polypeptide or a polynucleotide as claimed herein in the manufacture of a pharmaceutical or vaccine for immunotherapeutically treating a patient suffering from or susceptible to prostate cancer or other P501S-associated tumours or diseases.

FIGURE LEGENDS

Figure 1: amino acid sequence for rat P501S (SEQ ID N°1).

Figure 2: nucleotide sequence encoding rat P501S (SEQ ID N°2). The ORF appears in lower case.

Figure 3: amino acid sequence for Cynomolgus monkey P501S (SEQ ID N°3).

Figure 4: nucleotide sequence encoding Cynomolgus monkey P501S (SEQ ID N°4). The ORF appears in lower case.

Figure 5: amino acid sequence for human P501S (SEQ ID N°5).

Figure 6: amino acid sequence for human P501S (SEQ ID N°6).

Figure 7: amino acid sequence for human P501S (SEQ ID N°7).

Figure 8: design of the alpha prepro P501S His protein expressed in *Saccharomyces cerevisiae*.

Figure 9: amino acid sequence (SEQ ID NO:9) and nucleotide sequences (SEQ ID NO:8) of alpha prepro P501S his tailed recombinant protein expressed in Saccharomyces cerevisiae

Figure 10: Saccharomyces cerevisiae (strain Y1790) P501S-His fermentation process

Figure 11: Real-time PCR analysis of P501S on Cynomolgus prostate and on a panel of rat tissues and cell lines. Abbreviations are depicted in Table 1.

Figure 12: amino acid sequence for mouse P501S (SEQ ID N°10)

Figure 13: nucleotide sequence for mouse P501S (SEQ ID N°11). The ORF appears in lower case.

Figure 14: Real-time PCR analysis of P501S on a panel of mouse tissues.

The invention will be further described by reference to the following examples:

EXAMPLE I:

Preparation of recombinant Yeast strain *Saccharomyces cerevisiae* expressing alphaprepro P501S His tailed, under Cup1 promoter

1. – Introduction

The yeast expression system detailed below is suitable to express:

i) either recombinant non-human (monkey, rat, mouse for example) protein to be formulated subsequently in a vaccine or pharmaceutical composition to be inoculated into humans. Xenogeneic P501S can be expressed with its own signal sequence or with alpha prepro signal sequence (similarly to what is illustrated below).

ii) or recombinant human P501S protein to be formulated subsequently in a vaccine or pharmaceutical composition to be inoculated into animals (monkeys, rabbits, mouse or rat for example).

The Example below describes the expression of human P501S in yeast.

In order to target P501S protein in yeast endoplasmic reticulum (ER) membrane, the native secretion signal sequence and putative first lumenal domain was replaced by yeast alpha prepro signal sequence, in such a way that the natural position in membrane was conserved. The preparation of recombinant strain *Saccharomyces cerevisiae Y1790* expressing P501S as well as characterization of recombinant protein are described below.

2. – Protein design

The native secretion signal sequence and first putative lumenal domain of P501S protein was replaced by Saccharomyces cerevisiae alpha prepro signal sequence. The yeast signal sequence was fused to the N terminus of P501S sequence, coding from amino acid 55 to amino acid 553 (end of protein). The C terminal end of the recombinant protein was elongated by 2 glycines and six histidines (figure 8).

3. – Construction of pRIT15068 plasmid for Saccharomyces cerevisae expression

The starting material was the recombinant plasmid P501S, derived from commercial plasmid pcDNA3.1 (Invitrogen) containing a 3,4Kb insert between EcoRI and NotI cloning restriction sites. This plasmid contains the P501S full length coding sequence (1662 bp long) and was obtained from Corixa Corporation. The cloning strategy includes the following steps:

a. Subcloning of P501S:

A 1569 bp fragment containing nucleotide sequence coding for last 499 aminoacids + 68 bp in aval of the P501S open reading frame was isolated from Corixa p501S plasmid by Nco I digest. After T4 polymerase treatment, the fragment was subcloned in plasmid pUC18 opened by PstI and XbaI, T4 polymerase treated, in such a way that NcoI was recovered within the N terminal sequence of P501S open reading frame (i.e. amino acid position 55). The plasmid obtained was called pRIT15061.

b. Introduction of S. cerevisiae CUP1 promoter and yeast alpha prepro signal sequence:

A PCR fragment containing the yeast CUP1 promoter and yeast alpha prepro signal sequence was obtained by 3 successive PCR steps:

PCR step 1: the amplification of CUP1 promoter with oligonucleotides MDENHE1CUP1 (c 5' GGA CTA GTC TAG CTA GCT TGC TGT CAG TCA CTG TCA AGA G 3') and MDECUP1ATG (nc 5'CAT TTT ATG TGA TGA TTG ATT G 3') was performed on pRIT12471 plasmid as template.

pRIT12471 was obtained as follows: plasmid Yep6-36 harbouring the CUP-1 gene (Butt TR et al., Proc Natl Acad Sci U S A. 1984 Jun;81(11):3332-6) was received from TR. Butt (SmithKline Beecham Pharmaceuticals, Research and Development,

King of Prussia, Pennsylvania, USA). A BamHI-BbvI fragment (468 base pairs) containing the CUP-1 promoter and the N-terminal coding sequences was isolated from Yep6-36 plasmid, and treated with Bal31 enzyme in order to remove the N-terminal coding region and place a BamHI site adjacent to the ATG. After Bal31 treatment the DNA fragments were inserted into pAB119, a pBR322 like plasmid previously digested by BamHI and T4 polymerase repared. Several derivative plasmids were obtained and sequenced, amongst which pRIT12471.

PCR step 2: the amplification of alpha preprosignal sequence with oligonucleotides MDEPREPROAT (c 5'CAA TCA ATC AAT CAT CAC ATA AAA TGA GAT TTC CTT CAA TTT TTA CTG CA 3') and MDESIGNAL2 (nc5' GCT AGC TCC ATG GCT TCA GCC TCT CTT TTC TCG AG 3') was performed on pPIC9 plasmid (INVITROGEN) as template.

PCR step 3: the association of CUP1 promoter and alpha preprosignal sequence by PCR was performed using the fragments obtained by PCR step 1 and PCR step 2 and oligonucleotides MDENHE1CUP1 and MDESIGNAL2. After this step, the amplified fragment was purified, treated with T4 polymerase and digested by NcoI. The resulting fragment was introduced into plasmid pRIT15061 between the HindIII site treated with T4 polymerase, and the NcoI site. This resulting plasmid was called pRIT15062.

c. Elongation of the C terminus by HIS tail:

The fragment for HIS tail elongation was obtained by PCR using p501S plasmid as a template and oligonucleotides MDE501SAC (c 5'CTG GAG GTG CTA GCA GTG AG 3') and MDE501HIS (nc 5'CTA GTC TAG AGA ATT CCC CGG GTT AAT GGT GAT GGT GAT GGT GTC CAC CCG CTG AGT ATT TGG CCA AGT CG 3'). The amplified fragment was purified and digested by SacI and EcoRI and introduced between SacI (overlapping aminoacid 43) and EcoRI sites in pRIT 15062 plasmid, restauring correct open reading frame and elongating, in frame, p501S sequence by sequence coding for 2 glycines, 6 histidines, a stop codon . Moreover a SmaI site and EcoRI site are still introduced. This plasmid was called pRIT15063.

d. Introduction of promoter and coding sequence in yeast expression vector:

The FspI-SmaI fragment carrying the promoter and the recombinant P501S coding sequence was isolated from pRIT15063 plasmid and cloned in BamHI site, treated with T4 polymerase, of pRIT 15073 plasmid in such a way that the fragment was oriented with the C terminal of protein near the ARG3 terminator sequence. This last plasmid is a *E.coli/S. cerevisiae* shuttle vector carrying LEU2 gene for yeast complementation and the complete 2 micron sequence. This ligation leads to pRIT15067 plasmid.

e. An unexpected nucleotide deletion was found out in alpha prepro sequence, so the last step was performed to restore the sequence:

The full-length p501S coding sequence and the vector sequence were recovered from pRIT15067 plasmid on 2 fragments NcoI/SalI and SalI/NheI. A new fragment carrying CUP1 promoter and yeast alpha prepro signal sequence was isolated as described in step b and digested by NheI and NcoI. These 3 fragments were ligated together to obtain pRIT15068 expression plasmid. In this plasmid, P501S expression is driven by yeast CUP1 promoter.

The nucleotide (SEQ ID NO:8) and amino acid sequence (SEQ ID NO:9) of the recombinant protein are illustrated in figure 9.

4. – Transformation of *S. cerevisiae* DC5 strain and generation of Y1790 strain

The transformation of DC5 strain (a his3 leu 2-3 leu 2-112 can1-11) was performed by the lithium acetate method (Methods in enzymology, 1991, vol 194, pg 186) using plasmid pRIT15068. Yeast cells were spread on minimal medium plus histidine. Transformants were picked and tested for expression. Y1790 was one of these transformants.

5. – Induction of *S. cerevisiae* strain Y1790

Strain Y1790 was grown, at 30°C, in minimal medium supplemented with glucose 2% and histidine 80 ng/ml. Yeast cells were harvested in exponential growing phase and resuspended to a final OD = 0.5 in same medium supplemented with $CuSO_4$ to final concentration of 500 µg/ml for induction. Culture is maintained at 30°during 24h and then, cells are harvested for expression analysis.

EXAMPLE II:

Expression and characterization of recombinant p501S protein.

1. – Highlights

Using the process described below, the P501S antigen produced was clearly identified as a 62KD major band by Western Blot analysis. The antigen productivity was compared by WB analysis and densitometry. The antigen was located in the insoluble fraction obtained from the cell homogenate after centrifugation. The specific antigen productivity of strain Y1790 in fermenters was approximately 4 times higher than in flasks. As the biomass was amplified by a factor 10 in fermenter, the volumetric productivity was about 40 times higher in fermenter compared to flask cultures. Strain Y1790 (his-) was grown in fed-batch fermentation using 20 L vessels.

2. – Process description for strain Y1790 (see figure 10)

a. Pre-cultures

100µl of this lab Master Seed (MS) containing $2.5x10^8$ cfu /ml were spread on FSC004AA solid medium (see medium composition below). Two plates were incubated for 26h at 30°C. These solid pre-cultures were harvested in 5ml of liquid medium FSC007AA each and 0.5ml (or $9.3x10^7$ cells) of this suspension was used to inoculate each of the 2 liquid pre-cultures.

These pre-cultures were run for 20 hours in 2L flasks containing 400ml of medium FSC007AA in order to obtain an OD of 1.8. The other characteristics of these pre-cultures are the following : pH 2.8-glucose 2.3g/L-ethanol 3.4g/L.

The best timing for liquid pre-cultures for strain Y1790 was determined in preliminary experiments. Liquid pre-cultures containing 400 ml of medium and inoculated with various volumes of MS (0.25, 0.5, 1 and 2 ml) was monitored in order to identify the best inoculum size and timing for process. Glucose, ethanol, pH and OD and cell number (flow cytometry) were followed between 16 and 23 hours of culture. Glucose exhaustion and maximal biomass were obtained after 20 hour incubation with 0.5 inoculum. These conditions were adopted for transferring the pre-culture into fermentation.

a. Fermentation process

In total, 800ml of pre-culture were used to inoculate a 20 L fermenter containing 5L of medium FSC002AA. 3ml of irradiated antifoam were added before inoculation. The carbon source (glucose) was supplemented to the culture by a continuous feeding of the FFB004AA medium. The residual glucose concentration was maintained very low (≤50mg/L) in order to minimise the ethanol production by fermentation. This was realised by limiting the development of the micro-organism by limited glucose feed rate. The Standard biomass content (OD 80-90) for DC5 host strain was reached in fermentation after 44 hour growth phase.

CUP1 promoter was then induced by adding CuSO4 500µM in order to produce P501S antigen. CuSO4 addition was followed by ethanol accumulation (up to 6g/L), and glucose feeding rate was then reduced in order to consume the ethanol produced. The copper available for the micro-organism was monitored by testing Cu ion concentration in the broth supernatant using a spectrophotometric copper assay (DETC method).

The fermentation was then supplemented by CuSO4 throughout the induction phase in order to maintain its concentration between 150 and 250µM in the supernatant. The biomass reached an OD of 100 at the end of induction. Culture was harvested after 8 hours of induction.

c. Antigen characterisation and productivity

The cell homogenate was prepared and analysed by SDS-PAGE and Western Blot (WB) using standard protocols. A major protein band with the expected MW of 62KD was detected by WB using Corixa monoclonal P501S antibodies. WB analysis also showed that the major 62KD band was progressively produced from 30 minutes of induction on, and reached a maximum after 3 hours. No more antigen seemed to be produced between 3 and 12 hours of induction.

The number of passages through French Press necessary to extract all the antigen from the cells was evaluated. One, three and five passages were tested and total cell lysates, supernatants and pellets of cell lysates were analysed by WB. Three passages through French Press were sufficient to completely extract the antigen. Nothing was visible in

the supernatants, the antigen was associated to the insoluble fraction. A washing step will facilitate the purification by elimination of a part of the soluble proteins.

d. Culture media composition

FFB004AA

Glucose:350 g/l; Na2MoO4.2H2O:5.15 mg/l; Acide folique: 1.36 mg/l; KH2PO4: 20.6 g/l; MnSO4.H2O:10.3 mg/l; Inositol: 1350 mg/l; MgSO4.7H2O:11.7 g/l; H3BO3:12.9 m/l; Pyridoxine:170 mg/l; CaCl2.2H2O:2.35 g/l; KI:2.6 mg/l; Thiamine:170 g/l; NaCl:0.15 g/l; CoCl2.6H2O:2.3 mg/l; Niacine:0.67 mg/l; HCl:2.5 ml/l; FeCl3.6H2O:24.8 mg/l;Riboflavine:0.33 mg/l; CuSO4.5H2O:1.03 mg/l; Biotine:1.36 mg/l; Panthotenate Ca:170 mg/l; ZnSO4.7H2O:10.3 mg/l; Para-aminobenzoic acid: 0.33 mg/l; Histidine:5.35 g/l.

FSC007AA

Glucose:10 g/l; Na2MoO4.2H2O:0.0002 g/l; Acide folique:0.000064 g/l; KH2PO4:1 g/l;MnSO4.H2O:0.0004 g/l; Inositol:0.064 g/l; MgSO4.7H2O:0.5 g/l; H3BO3:0.0005 g/l;Pyridoxine:0.008 g/l; CaCl2.2H2O:0.1 g/l; KI:0.0001 g/l; Thiamine:0.008 g/l; NaCl:0.1 g/l; CoCl2.6H2O:0.00009 g/l; Niacine:0.000032 g/l; FeCl3.6H2O:0.0002 g/l; Riboflavine:0.000016 g/l; Panthotenate Ca:0.008 g/l; CuSO4.5H2O:0.00004 g/l; Biotine:0.000064 g/l; para-aminobenzoic acid:

FSC002AA

(NH4)2SO4:6.4 g/l; Na2MoO4.2H2O: 2.05 mg/l; Acide folique: 0.54 mg/l; KH2PO4:8.25 g/l; MnSO4.H2O:4.1 mg/l; Inositol:540 mg/l; MgSO4.7H2O:4.69 g/l; H3BO3:5.17 m/l; Pyridoxine:68 mg/l; CaCl2.2H2O:0.92 g/l; KI:1.03 mg/l; Thiamine:68 mg/l; NaCl:0.06g/l; CoCl2.6H2O:0.92mg/l; Niacine:0.27 mg/l; HCl:1 ml/l; FeCl3.6H2O:9.92 mg/l; Riboflavine:0.13 mg/l; CuSO4.5H2O:0.41 mg/l; Glucose:0.14 g/l; Panthotenate Ca:68 mg/l; ZnSO4.7H2O:4.1 mg/l; Biotine:0.54 mg/l; para-aminobenzoic acid: 0.13 mg/l; Histidine:0,3 g/l.

FSC004AA

Glucose:10 g/l; Na2MoO4.2H2O:0.0002 g/l; Acide folique: 0.000064 g/l; KH2PO4: 1 g/l;MnSO4.H2O:0.0004 g/l; Inositol:0.064 g/l; MgSO4.7H2O:0.5 g/l;

H3BO3:0.0005 g/l; Pyridoxine:0.008 g/l; CaCl2.2H2O:0.1 g/l; KI:0.0001 g/l; Thiamine:0.008 g/l; NaCl: 0.1 g/l; CoCl2.6H2O:0.00009 g/l; Niacine:0.000032 g/l; FeCl3.6H2O:0.0002 g/l; Riboflavine:0.000016 g/l; Panthotenate Ca:0.008 g/l; CuSO4.5H2O: 0.00004 g/l; Biotine:0.000064 g/l; para-aminobenzoic acid:0.000016 g/l; ZnSO4.7H2O:0.0004 g/l; (NH4)2SO4: 5 g/l; Agar 18 g/l; Histidine:0.1 g/l.

EXAMPLE III:

Compositions and methods to induce an immune response

A - VACCINE PREPARATION USING XENOGENEIC OR HUMAN P501S

1. – Vaccine preparation:

The vaccine used in these experiments is produced from a recombinant DNA, encoding a human or xenogeneic P501S recombinantly expressed in *S. cerevisiae*, either adjuvanted or not. As an adjuvant, the formulation comprises a mixture of 3 de -O-acylated monophosphoryl lipid A (3D-MPL) and QS21 in an oil/water emulsion. The adjuvant system SBAS2 has been previously described WO 95/17210.

3D-MPL: is an immunostimulant derived from the lipopolysaccharide (LPS) of the Gram-negative bacterium *Salmonella minnesota*. MPL has been deacylated and is lacking a phosphate group on the lipid A moiety. This chemical treatment dramatically reduces toxicity while preserving the immunostimulant properties (Ribi, 1986). Ribi Immunochemistry produces and supplies MPL to SB-Biologicals. Experiments performed at Smith Kline Beecham Biologicals have shown that 3D-MPL combined with various vehicles strongly enhances both the humoral and a TH1 type of cellular immunity.

QS21: is a natural saponin molecule extracted from the bark of the South American tree Quillaja saponaria Molina. A purification technique developed to separate the individual saponines from the crude extracts of the bark, permitted the isolation of the particular saponin, QS21, which is a triterpene glycoside demonstrating stronger adjuvant activity and lower toxicity as compared with the parent component. QS21 has been shown to activate MHC class I restricted CTLs to several subunit Ags, as well as to stimulate Ag specific lymphocytic proliferation

(Kensil, 1992). Aquila (formally Cambridge Biotech Corporation) produces and supplies QS21 to SB-Biologicals.

Experiments performed at SmithKline Beecham Biologicals have demonstrated a clear synergistic effect of combinations of MPL and QS21 in the induction of both humoral and TH1 type cellular immune responses.

The oil/water emulsion is composed an organic phase made of of 2 oils (a tocopherol and squalene), and an aqueous phase of PBS containing Tween 80 as emulsifier. The emulsion comprised 5% squalene 5% tocopherol 0.4% Tween 80 and had an average particle size of 180 nm and is known as SB62 (see WO 95/17210).

Experiments performed at SmithKline Beecham Biologicals have proven that the adjunction of this O/W emulsion to 3D-MPL/QS21 (SBAS2) further increases the immunostimulant properties of the latter against various subunit antigens.

2. – Preparation of emulsion SB62 (2 fold concentrate):

Tween 80 is dissolved in phosphate buffered saline (PBS) to give a 2% solution in the PBS. To provide 100 ml two fold concentrate emulsion 5g of DL alpha tocopherol and 5ml of squalene are vortexed to mix thoroughly. 90ml of PBS/Tween solution is added and mixed thoroughly. The resulting emulsion is then passed through a syringe and finally microfluidised by using an M110S microfluidics machine. The resulting oil droplets have a size of approximately 180 nm.

3. – Preparation of xenogeneic or human P501S QS21/3D MPL oil in water (SBAS2) formulation:

The adjuvant is formulated as a combination of MPL and QS21, in an oil/water emulsion. The formulations are prepared extemporaneously on the day of injection.

The formulations containing 3D-MPL and QS21 in an oil/water emulsion (SBAS2B formulations) are performed as follows: xeno or human P501S (20µg) is diluted in 10-fold concentrated PBS pH 6.8 and H_2O before consecutive addition of SB62 (50µl), MPL (20µg), QS21 (20µg) and 1 µg/ml thiomersal as preservative at 5 min intervals. All incubations are carried out at room temperature with agitation.

The non-adjuvanted formulations are performed as follows: recombinant xeno P501S (20µg) is diluted in 1.5 M NaCl and H_2O before addition of 1 µg/ml thiomersal as preservative at 5 min intervals. All incubations are carried out at room temperature with agitation.

B – IMMUNOGENICITY EXPERIMENTS

Immunisation protocol

A xenogeneic antigen to human P501S can be used, according to the present invention, to induce an immune response against a closely related autologous tumour antigen. Similarly a human P501S can be used to immunise animal species and assess the level of cross-reacting antibodies. The quality and the intensity of the immune response induced by different molecules can be compared as well as the capacity of this immune response to cross- react with other forms of the P501S protein. The protein can be adjuvanted or not.

Rabbits were vaccinated three times, intramuscularly, at 3 weeks interval with 100 µg of human P501S formulated or not in SBAS02 (see above). Three weeks after the third injection, blood can be taken and the sera tested for the presence of anti-P501S antibodies.

The anti-P501S antibody response (Total IgG Antibody response) is classically assessed by ELISA, using purified human P501S protein as a coating antigen.

Spleen and lymph nodes of these immunized animals can also be used to analyze the cellular immune responses induced by the vaccinations. Lymphoproliferative responses can be evaluated after 72 hours of in-vitro re-stimulation with the different forms of the molecules used to vaccinate, or with the purified human P501S protein.

EXAMPLE IV:

Analysis of P501S expression by real-time PCR

1. – Introduction

Expression analysis of the P501S will be done in animal models and in animal cell lines by monitoring the P501S mRNA abundance by real-time PCR.

Animal models are used to test vaccine composition and to evaluate their immunogenicity (ex: specific CTL induction) and their potential toxicity (ex: autoimmunity). The more relevant animal model will display a tissue expression pattern of the P501S, which is the closest to the human profile. Expression level measurement will be done in animal prostate and in a panel of essential tissues.

Real-time PCR is also used to characterise the expression level of the P501S gene in animal cell lines such as rat prostate cell lines (CRL-2275, CRL-2276). Objective being to identify animal cell lines, which are expressing P501S at a level, which is closest to the level observed in human prostate tumours. Animal cell lines identified to express reasonable level of P501S mRNA could be used to establish an animal tumour model. Anti-tumour effects of vaccination using the P501S-purified protein in adjuvant could be monitored either by tumour regression or by protection against tumour challenge in the animal.

2. – Real-time RT-PCR analysis

Real-time RT-PCR (U. Gibson. 1996. Genome Research: 6,996) is used to compare mRNA transcript abundance of the target protein in a panel of tissues and cell lines.

Total RNA is extracted from snap frozen biopsies using TriPure reagent (Boehringer). Poly-A+ mRNA is purified from total RNA after DNAase treatment using oligo-dT magnetic beads (Dynal). Quantification of the mRNA is performed by spectrofluorimetry (VersaFluor, BioRad) using RiboGreen (Molecular Probes). Primers for real-time PCR amplification are designed with the Perkin-Elmer Primer Express software using default options for TaqMan amplification conditions.

Real-time reactions are assembled according to standard PCR protocols using 2 ng of purified mRNA for each reaction. Real-time PCR amplification are monitored using a Taqman probe. Amplification (40 cycles) and real-time detection is performed

in a Perkin-Elmer Biosystems PE7700 system using conventional instrument settings. Ct values are calculated using the PE7700 Sequence Detector Software. Ct values are obtained from each tissue sample for the target mRNA (CtX) and for the beta actin mRNA (CtA).

As the efficiency of PCR amplification under the prevailing experimental conditions is close to the theoretical amplification efficiency, $2^{(CtA-CtX)}$ value is an estimate of the relative target transcript level in the sample, standardized with respect to Actin transcript level. A value of 1 thus suggests that the candidate antigen and Actin have the same expression level.

For the rat model, real-time (RT) PCR reactions were performed on 2 rat prostate cell lines (CRL-2222 and CRL-2276) and on a panel of 11 rat tissues such as brain, colon, femur, gum, heart, kidney, liver, lung, prostate, spleen, testis.

For the Cynomolgus model, expression of P501 homologue was evaluated in prostate.

For the mouse model, expression level was determined in prostate, colon, lung, brain, kidney, spleen, testis, stomach, heart and liver.

P501 homologue transcript level are calculated as described above. Results are shown in Table 1, Table 2, Table 3 and figures 11 and 14.

Table 1: RT-PCR analysis of P501S on a panel of rat tissues and rat cell lines.

P501 expression analysis in rat tissues				
Tissue	**Abbreviation**	**CT of P501S**	**CT of actin**	**P501S Actin**
prostate	Pr	26	19	8.2E-03
brain	Bra	34	19	3.1E-05
colon	Co	31	18	1.4E-04
kidney	Ki	31	19	1.4E-04
lung	Lu	35	18	1.4E-05
testis	Te	37	20	9.3E-06
gum	Gu	32	18	7.9E-05
spleen	Sp	30	17	9.7E-05
heart	He	35	21	5.5E-05
liver	Lu	33	20	9.2E-05
tumor cell 2222	TC2222	34	17	8.3E-06
tumor cell 2276	TC2276	40	17	8.7E-08
femur	Fe	31	17	6.3E-05

Table 2: RT-PCR analysis of P501S on Cynomolgus prostate.

P501 expression analysis in Cynomolgus prostate				
Tissue	**Abbreviation**	**CT of P501S**	**CT of actin**	**P501S Actin**
prostate	Pr	24	19	5.3E-02

Table 3: RT PCR analysis of P501S on a panel of 10 mouse tissues

P501 expression analysis in mouse tissues							
	Exp. 1		Exp. 2		Exp. 3		P501S Actin
Tissue	CT of P501S	CT of actin	CT of P501S	CT of actin	CT of P501S	CT of actin	
prostate	24	19	27	20	26	20	**1.5E-02**
colon	27	18	30	19	31	21	**1.2E-03**
lung	28	18	33	20	33	21	**3.9E-04**
brain	30	20	34	21	36	23	**3.3E-04**
kidney	28	19	31	22	31	22	**2.6E-03**
spleen	29	18	31	20	31	20	**5.6E-04**
testis	29	18	32	19	33	20	**2.7E-04**
stomach	28	19	31	21	31	22	**1.1E-03**
heart	32	21	33	23	35	24	**6.7E-04**
liver	29	21	31	23	32	24	**5.5E-03**

P501S is expressed in rat, Cynomolgus and mouse prostate (0.8%, 5.3% and 1.5% relative to actin level, respectively). Average P501 trancript level in rat other tissues (0.007%) is hundred fold lower than in rat prostate. No significant expression was detected in both rat cell lines. In other mouse tissues, the highest expression level was detected in the liver and in the kidney (3 and 6 times lower than in mouse prostate, respectively).

EXAMPLE V:

Induction of P501S-specific CD4 or CD8 T cells by xenogeneic P501S

A T-cell in vitro priming protocol is used to demonstrate the capacity of the human immune repertoire to recognize the P501S protein as a potential target for

immunotherapy. This protocol can be used to generate and expand either CD4 or CD8 human T cells that specifically recognise either the P501S -derived peptide or the P501S protein loaded onto targets but also human cells that endogeneously express the P501S.

The protocol used to generate P501S specific CD8 T cells is briefly described: Human dendritic cells (DC) genetically engineered to express the xenogeneic P501S gene or pulsed with 1 μg/ml xenogeneic P501S -derived peptides, are matured for 48 hours using CD40L, and cultured with autologous PBMC in medium supplemented with IL-7. Weekly stimulations are performed using adherent PBMC pulsed with 1 μg/ml xenogeneic P501S, with the addition of IL-7 on day 0 and 4, and IL-2 on days 1 and 4. Lines are assayed following the 4^{th}, 5^{th}, and 6^{th} round of stimulation by ELISPOT assays to measure IFNg secretion. Antigen presenting cells (APC) in the ELISPOT assays are autologous B-LCL, pulsed either with the xenogeneic P501S or an irrelevant peptide. Specific CTL activity is initially detectable after the 5^{th} or the 6^{th} stimulation cycles against xenogeneic P501S pulsed or transduced APC.

A similar protocol can be used to generate P501S specific CD4 T cell clones.

CLAIMS

1. A pharmaceutical composition comprising a xenogeneic P501S polypeptide or a xenogeneic P501S-encoding polynucleotide; and a pharmaceutically acceptable carrier.

2. A pharmaceutical composition as claimed in claim 1 wherein the xenogeneic P501S polypeptide is selected from the group comprising SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:10.

3. A pharmaceutical composition as claimed in claim 1 wherein the xenogeneic P501S-encoding polynucleotide is selected from the group comprising SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:11.

4. A pharmaceutical composition as claimed in any of claims 1 to 3 which additionally comprises a TH-1 inducing adjuvant.

5. A pharmaceutical composition as claimed in claim 4 in which the TH-1 inducing adjuvant is selected from the group of adjuvants comprising: 3D-MPL, QS21, an immunostimulatory CpG oligonucleotide, a mixture of QS21 and cholesterol or a combination of one or more of any of these adjuvants.

6. A vaccine comprising an effective amount of antigen presenting cells, modified by in vitro loading with a xenogeneic P501S polypeptide, or genetically modified in vitro to express a xenogeneic P501S polypeptide and a pharmaceutically effective carrier.

7. A pharmaceutical composition as claimed in any of claims 1 to 6 for use in medicine.

8. A process for the production of a pharmaceutical composition as claimed in any of claims 1 to 7, comprising admixing a xenogeneic P501S polypeptide or a xenogeneic P501S-encoding polynucleotide with a suitable adjuvant, diluent or other pharmaceutically acceptable carrier.

9. An isolated polypeptide comprising an amino acid sequence which has at least 92% identity to the amino acid sequence of SEQ ID NO:1 over the entire length of of SEQ ID NO:1.

10. An isolated polypeptide as claimed in claim 9 in which the amino acid sequence has at least 95% identity to SEQ ID NO:1.

11. The polypeptide as claimed in claim 10 comprising the amino acid sequence of SEQ ID NO:1.

12. The isolated polypeptide of SEQ ID NO:1.

13. A polypeptide comprising an immunogenic fragment of a polypeptide as claimed in any one of claims 9 to 12 in which the immunogenic activity of the immunogenic fragment is substantially the same as the polypeptide of SEQ ID NO:1.

14. A polypeptide as claimed in any of claims 9 to 13 wherein said polypeptide is part of a larger fusion protein.

15. An isolated polynucleotide encoding a polypeptide as claimed in any of claims 9 to 14.

16. The isolated polynucleotide of claim 15, comprising the sequence of SEQ ID NO:2.

17. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 92% identity to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

18. The isolated polynucleotide as defined in any one of claims 15 to 17 in which the identity is at least 95%.

19. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 15 - 18.

20. A host cell comprising the expression vector of claim 19 or the isolated polynucleotide of claims 15 to 18.

21. A process for producing a polypeptide of claims 9 to 14 comprising culturing a host cell of claim 20 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

22. The use of a polypeptide or a polynucleotide as claimed in any of claims 9 to 18 in the manufacture of a vaccine for immunotherapeutically treating a patient suffering from or susceptible to prostate cancer or other P501S-associated tumours or diseases.

23. A method of inducing an immune response against human P501S having an amino acid sequence as set forth in SEQ ID NO:5 to SEQ ID NO:7 in a human, comprising administering to the subject an effective dosage of a pharmaceutical composition comprising a xenogeneic form of said human P501S.

24. The method of claim 23, wherein said pharmaceutical composition is according to any of claims 1 to 5.

25. The method of claim 23, wherein said xenogeneic form of human P501S is the rat P501S as claimed in any of claims 9 to 14.

26. The method of claim 23, wherein said xenogeneic form of human P501S is selected from the group consisting of the mouse P501S having the sequence as set forth in SEQ ID NO:10 and the Cynomolgus monkey P501S having the sequence set forth in SEQ ID NO:3.

27. The method of any of claims 23 to 26, wherein said antigen composition includes a live viral expression system or a plasmid vector which expresses said xenogeneic antigen, ot through antigen loaded dendritic cells.

ABSTRACT

Vaccine

The present invention relates to pharmaceutical compositions and methods for inducing an immune response against tumours-related antigens. More specifically, the invention relates to non-human prostate-specific antigens, more precisely to the non-human prostate-specific P501S, which can be used as xenogeneic antigen in prostate cancer vaccine therapy and as diagnostic agents for prostate tumours in humans, to pharmaceutical compositions containing them, to methods of manufacture of such compositions and to their use in medicine. Methods for formulating vaccines for immunotherapeutically treating P501S-expressing prostate tumors, prostatic hyperplasia, and prostate intraepithelilial neoplasia (PIN) are also provided.

FIG. 1: amino acid sequence for rat P501S (SEQ ID N°1)

MIQRLWASRLLRHRKAQLLLVNLLTFGLEVCLAAGITYVPPLLLEVGVEEKFMTMVL
GIGPVLGLVSVPLLGSASDQWRGRYGRRRPFIWALSLGVLLSLFLIPRAGWLAGLLC
SDTRPLELALLILGVGLLDFCGQVCFTPLEALLSDLFRDPDHCRQAFSVYAFMISLG
GCLGYLLPAIDWDTSALAPYLGTQEECLFGLLTLIFLICVAATLLVAEEAVLGPPEP
AEGLLVSSVSRRCCSCHAGLAFRNLGTLFPRLHQLCCRMPRTLRRLFVAELCSWMAL
MTFTLFYTDFVGEGLYQGVPRAEPGTEARRHYDEGIRMGSLGLFLQCAISLFFSLVM
DRLVQKFGTRSVYLASVMTFPVAAAATCLSHSVVVVTASAALTGFTFSALQILPYTL
ASLYHREKQVFLPKYRGDAGGGSSEDSQTTSFLLGPKPGAPFPNGHVGPGGSSILVP
PPALCGASACDVSMRVVVGEPPEAKVVTGRGICLDLAILDSAFLLSQVAPSLFMGSI
VQLSHSVTAYMVSAAGLGLVAIYFATQVVFDKNDLAKYSL

FIG.2: nucleotide sequence encoding rat P501S (SEQ ID N°2)

GGGCTCTTAGACACCGCAACAAAAGCAACTTTCCTCCAAGCCACTGCCACCTGTTGG
GTTTTCACACATTTCGAGCTTTAGTTCCGATCCCCAGAACATCCACGTAGTTTTTCT
GGCCTTCTGGCTGAGCCATGGAGGCCGACAGAGGAGGGGAGAAGTTTGAAGCTTGAG
AAGGATTTCCGTATGCGCAAGGCTACCCATGCTTGTCCTTCCTCCCATGACCCTGGT
CAGCCCTCCTCTGCCCTCCTCTTCCTGCCCCCCTTCTCTCCAGGGTCCGACTGACGA
GATGTGTCCCCATCAAGCAAGGCACTAGATGGTGACGTGTTCAGTGTGGGATGAGAT
GCCGAAGTGGTACTCAAGGGCTGGCCGAAATGGGAGCCTGGCTGCACCCTCGGAGGT
TGGTGCTAGCAAGGAGGAGAAGCCGCGGCAGGGCTGACTCAAAACAGCTGTGGGGTG
TGTGAATGGCCCCCGGACCCCTAACCGCCCTGTCCATCatgatccagaggctgtggg
ccagccgtctgctaaggcatcggaaagcccagctcctgctggtcaacctgctaacct
tcggcctggaggtgtgcctggctgctggcattacctatgtgccacccttctgctgg
aagtcggggtagaggaaaagttcatgaccatggtgttgggcattggcccagtgctgg
gcctggtttctgttccactcctaggctcagccagtgaccagtggcgtgggcgctatg
gccgccggagacccttatctgggctctgtccctgggtgtcctgctaagcctcttcc
tcatcccgagggccggctggctggcagggctactgtgttcagatactaggcccctgg
agttggccctgctcatcttgggagtggggctgctggacttttgcggccaggtgtgct
ttactccactggaggccttactctccgacctcttccgggacccagaccactgccgcc

aagccttctctgtctatgccttcatgatcagcctcgggggctgcctgggctacctct
tacctgccattgactgggacaccagcgccctggccccctacctaggcactcaggaag
aatgcctcttcggcctcctcaccctcatttttctcatctgtgtggcagccactctgc
ttgtggctgaggaggcagtccttggcccacccgagccagcagaagggttgttggtct
cctccgtgtcacgccggtgctgctcatgccatgctggcctggctttccggaatctgg
gtaccctgtttccccggctgcaccagctgtgctgccgaatgcctcgcaccctgcgcc
ggctctttgtggctgagctgtgcagctggatggcacttatgactttcacactgttct
acacggacttcgtgggagaggggctgtaccagggtgtccccagagcagagccaggta
ccgaggcccggagacactatgatgaaggcattcgaatgggcagcctggggctcttcc
tgcagtgtgccatctccctgttcttctccctggtcatggacaggctggtacagaagt
tcggcacacggtcagtctacctggccagtgtgatgacctttcccgtggctgccgctg
ccacgtgcctgtcccacagcgtggttgtagtgacagcctcagctgccctcaccgggt
tcaccttctcagccttgcagatcctgccttacacgctcgcctccctctaccatcgag
agaagcaggtgttcctgcccaaataccgaggggacgctggaggtggtagcagtgaag
acagccaaacaaccagcttcttgctaggccctaagccaggagctcccttccccaatg
gacacgtgggccctggcggcagcagcatcctggtgccccacctgcactctgtgggg
cctctgcctgtgatgtctccatgcgagtggtagtgggtgagccacctgaagccaagg
ttgttactggacggggcatttgcctggaccttgccatcctggacagtgcctttctgc
tgtcccaggtggctccgtccctgttcatgggctccattgtccagctgagccactctg
tcactgcctatatggtatcagctgcaggcttgggtctggtcgccatttactttgcta
cacaggtagtgtttgacaagaatgacttggccaaatactcactgtagAATTCTGTAA
GGCATCAAAGAAGAGGATCTGCCTCCCCGGTTCTCAGCCCCAGAGGGCTGCAGAGCT
GGTCTCTTTCCGGTCTCTGTTGCCCTGAGTGGCTCTCCACTGCCATCCGAAGGCAGT
GAGGTGTATGGCTGCACAGGTTGGAGCTTTTGGC

FIG. 3: amino acid sequence for Cynomolgus monkey P501S (SEQ ID N°3)

MVQRLWVSRLLRHRKAQLLLINLLTFGLEVCLAAGITYVPPLLLEVGVEEKFMTMVL
GIGPVLGLVSVPLLGSASDHWRGRYGRRRPFIWALSLGILLSLFLIPRAGWLAGLLC
PDPRPLELALLILGVGLLDFCGQVCFTPLEALLSDLFRDPDHCRQAYSVYAFMISLG
GCLGYLLPAIDWDTSALAPYLGTQEECLFGLLTLIFLTCVAATLLVAEEAALGPAEP
AEGLSAPSLPSHCCPCWARLAFRNLGALLPRLHQLCCRMPRTLRRLFVAELCSWMAL
MTFTLFYTDFVGEGLYQGVPRAELGTEARRHYDEGVRMGSLGLFLQCAISLVFSLVM
DRLVQRFGTRAVYLASVAAFPVAAGATCLSHSVAVVTASAALTGFTFSALQILPYTL

ASLYHRERQVFLPKYRGDAGGTSSEDSLMTSFLPGPKPGAPFPNGHVGAGGSGLLPP
PPALCGASACDVSVRVVVGEPTEARVVPGRGICLDLAILDSAFLLSQVAPSLFMGSI
VQLSQSVTAYMVSAAGLGLVAIYFATQVVFDKSDLAKYSV

FIG.4: nucleotide sequence for Cynomolgus monkey P501S (SEQ ID N°4)

AAAAAAAAAGCCGCCGGCTGGCGCGCGTGGGGGGCAAGGAAAAGAGGGGGGAAACCA
GTCTGCACGCGCTGGCTCCGGGTGACAGCCGCGCGCCTAGGCCAGGCAGCGTCTCCC
TCTGTCACCCAGACTGGAGGCAATGTTCTGATCACTGCACACTGCACCCTTGACCTC
CCAGACTCAAGCAATCCTCCCATCTCAGCCTCTTAAGTAGCTGGGACCACAGGATCT
GAGTGATGAGATGTGTCCCCACTGAGGTGCCCCACAGCAGCAGGTGTTGAGCATGGG
CTGAGAAGCTGGACCGGCACCAAAGGGCTGGCGGAAATGGGCGCCTGGCTGATTCCT
AGGCAGTTGGCGGCAGCAAGGAGGAGAGGCCGTGGCTTCCGGAGCAGAGCGGAGACG
AAGCAGTTCTGGAGTGCTTAAACGGCCCCCTGAGCCCTACCCGCCTGGCCCACTatg
gtccagaggctgtgggtgagccgcctgctgcggcaccggaaagcccagctcttgttg
atcaacctgctaacctttggcctggaagtgtgtttggccgcaggcatcacctatgtg
ccgcctctgctgctggaagtgggggtagaagagaagttcatgaccatggtgctgggc
atcggtccagtgctgggcctggtctctgtcccactcctaggctcagccagtgaccac
tggcgcggacgctatggccgccggcggcccttcatctgggcgctgtccttgggcatc
ctgctgagcctctttctcatcccaagggctggctggctggcagggctgctgtgcccg
gatcccaggcccctggagctggcactgctcatcctgggcgtagggctgctggacttc
tgtggccaggtgtgcttcactccactggaggccctgctctctgacctgttccgggac
ccggaccactgtcgccaggcctactccgtctatgccttcatgatcagtcttgggggc
tgcctgggctacctcctgcctgccattgactgggacaccagtgccctggcccctac
ctgggcacccaggaggagtgcctctttggcctgctcaccctcatcttcctcacctgc
gtagcagccacactgctggtggccgaggaggcagcactgggccccgccgagccagcg
gaagggctgtctgccccctccctgccgtcccactgctgtccgtgctgggcccgcctg
gctttccggaacctgggcgccctgcttccccggctgcaccagctgtgctgccgcatg
ccccgcaccctgcgccggctcttcgtggctgagctgtgcagctggatggcactcatg
accttcacgctgttttacacggatttcgtgggcgaggggctataccagggcgtgccc
agagctgagctgggcaccgaggcccggagacactatgatgaaggcgttcggatgggc
agtctggggctgttcctgcagtgcgccatctccctggtcttctctctggtcatggac
cggctggtgcagcgattcggcactcgagcagtctatctggccagtgtggcagctttc
cctgtggctgccggtgccacgtgcctgtcccacagtgtggctgtggtgacggcttca

gccgccctcactgggttcaccttctcagccctgcagatcctgccctacacactggcc
tccctctaccaccgggagaggcaggtgttcctgcccaaataccgaggggacgctgga
ggcactagcagtgaggacagcctgatgactagcttcctgccaggccctaagcctgga
gctcccttccctaatggacacgtgggtgctggaggcagtggcctgcttccacctcca
cccgcgctctgcggggcctctgcctgcgatgtctctgtacgtgtggtggtgggtgag
cccaccgaggccagggtggttccgggccggggcatctgcctggacctcgccatcctg
gatagtgccttcctgctgtcccaggtggccccgtccctgttcatgggctccatcgtc
cagctcagccagtctgtcactgcctatatggtgtctgctgcaggcctgggtctggtt
gccatttactttgctacacaggtagtatttgacaagagcgacttggccaaatactcg
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CCCACTCTTTGTTAGCCCCATGGGGCTGCTGGGCTGGCCGCCAGTTTCTGTTGCTGC
CAAAGTAATGTGGCTCTCTGCTGCCACCCTGTGCTGCTGAGGTGCGTAGCTGCACAG
CTGGGGGCTGGGGCATCCCTCTCCCTCCTCCCCAGTCTCTAGGGCTGCCTGACTGGA
AGCCTTCCAAGGGGGTTTCAGTCTGGACTTCTACAGGGAGGCTAGAAGGGCAGGGCA
TTTGATTCGCTCCATGCACTGGAATGTGGGGACTCTGCAGGTGGATTACCCAGGCTC
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GAGTAAACTCAGTCACCTGGTTTCCCACCTCTAAGCCCCCTTAACCTGCAGCTTCAT
TTAATGTAGCTCTTGCATGGGAGTTTCTAGGATGAAACACTCCTCCGTGGGATTTGA
ACGTATGAAAGTTATTTGTAGGGGAAGAGTCCTGAGGGGCAACACACCAGGTCCCCT
CAGCCCACAGCACTGCCTTTTTGCTGATCCCCTGACTCTTACCTTTTATCAGGACGT
GGCCTATTGGTCCCTTTGTTGCCATCATAGGGACACAGGCATTTAAATATTTAACTT
ATTTATTTAACAAAGTAGAAGGGAATCCATTGCTAGCTTTTGTGTGTTGGTGTCTAA
GATTTGGGTAGGGTGGGATCCCCAACAATCAGGTCCACTGAGATCACTGGTCATTGG
GCTGATCATTGCCAGAATCTTCTTCTCCTGGGGTCTGGCTCCTCAAAATGCCTAACC
CAGGACCTTGGAAATTTTACTCATCCCGACTGATAATTCCAAATGCTGTTACCCAAG
GTTAGGGGGTTGAAGGAAGGTGGAGGGTGGGGCTTCAGGTCTCAACAGCTTCCCTAA
CCACCCCTTTTCTCTTGGCCCAGCCTGGTTCCCCCCACTTCTACTCCCCTCTACTGT
CTCTAGGACTGGGCTGATGAAGGCACTGCCTGAAATTTCCCTCACCCCCAACTTTCC
CCACTGGCTCCACAACCCTGTTTGGAGCTGTTGCAGGACCAGAAGCACAAAGTGTGG
TTTCCCAGGCCTTTGTCCATCTCAGCCCCCCAGAGTATATCTGTGCTTGGGGAATCT
CACACAGAAACTCAGGAGCACCCCCTGCCTGAGCTAAGGAGGTCTTATCTCTCAGGG
GGGTTTAAGTGCCGTTTGCAATAATGTCTTATTTATTTAGCGGGGCAAATATTTTAT
ACTGTAAGTGAGCAATCAGTATAATGTTTATGGTGATGAAATTAAAGGCTTTCTTAT
ATGTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIG.5: amino acid sequence for human P501S (SEQ ID N°5).

MVQRLWVSRLLRHRKAQLLLVNLLTFGLEVCLAAGITYVPPLLLEVGVEEKFMTMVL
GIGPVLGLVCVPLLGSASDHWRGRYGRRRPFIWALSLGILLSLFLIPRAGWLAGLLC
PDPRPLELALLILGVGLLDFCGQVCFTPLEALLSDLFRDPDHCRQAYSVYAFMISLG
GCLGYLLPAIDWDTSALAPYLGTQEECLFGLLTLIFLTCVAATLLVAEEAALGPTEP
AEGLSAPSLSPHCCPCRARLAFRNLGALLPRLHQLCCRMPRTLRRLFVAELCSWMAL
MTFTLFYTDFVGEGLYQGVPRAEPGTEARRHYDEGVRMGSLGLFLQCAISLVFSLVM
DRLVQRFGTRAVYLASVAAFPVAAGATCLSHSVAVVTASAALTGFTFSALQILPYTL
ASLYHREKQVFLPKYRGDTGGASSEDSLMTSFLPGPKPGAPFPNGHVGAGGSGLLPP
PPALCGASACDVSVRVVVGEPTEARVVPGRGICLDLAILDSAFLLSQVAPSLFMGSI
VQLSQSVTAYMVSAAGLGLVAIYFATQVVFDKSDLAKYSA

FIG.6: amino acid sequence for human PS108 (SEQ ID N°6).

GLYQGVPRAEPGTEARRHYDEGVRMGSLGLFLQCAISLVFSLVMDRLVQRFGTRAVY
LASVAAFPVAAGATCLSHSVAVVTASAALTGFTFSALQILPYTLASLYHREKQVFLP
KYRGDTGGASSEDSLMTSFLPGPKPGAPFPNGHVGAGGSGLLPPPPALCGASACDVS
VRVVVGEPTEARVVPGRGICLDLAILDSAFLLSQVAPSLFMGSIVQLSQSVTAYMVS
AAGLGLVAIYFATQVVFDKSDLAKYSA

FIG.7: amino acid sequence for human Y54369 (SEQ ID N°7).

MGSLGLFLQCAISLVFSLVMDRLVQRFGTRAVYLASVAAFPVAAGATCLSHSVAVVT
ASAALTGFTFSALQILPYTLASLYHREKQVFLPKYRGDTGGASSEDSLMTSFLPGPK
PGAPFPNGHVGAGGSGLLPPPPALCGASACDVSVRVVVGEPTEARVVPGRGICLDLA
ILDSAFLLSQVAPSLFMGSIVQLSQSVTAYMVSAAALGLVAIYFATQVVFDKSDLAK
YSA

FIG. 8: Design of the yeast P501S recombinant protein

FIG. 9: Amino acid and nucleotide sequence of alpha prepro P501S his tailed recombinant protein expressed in Saccharomyces cerevisiae

Nucleotide sequence (SEQ ID NO:8)

ATGAGTTTCC TCAATTTTAC TGCAGTTTTA TTCGCAGCAT CCTCCGCATT
AGCTGCTCCA GTCAACACTA CAACAGAAGA TGAAACGGCA CAAATTCCGG
CTGAAGCTGT CATCGGTTAC TCAGATTTAG AAGGGGATTT CGATGTTGCT
GTTTTGCCAT TTTCCAACAG CACAAATAAC GGGTTATTGT TTATAAATAC
TACTATTGCC AGCATTGCTG CTAAAGAAGA AGGGGTATCT CTCGAGAAAA
GAGAGGCTGA AGCCatgGTG CTGGGCATTG GTCCAGTGCT GGGCCTGGTC
TGTGTCCCGC TCCTAGGCTC AGCCAGTGAC CACTGGCGTG GACGCTATGG
CCGCCGCCGG CCCTTCATCT GGGCACTGTC CTTGGGCATC CTGCTGAGCC
TCTTTCTCAT CCCAAGGGCC GGCTGGCTAG CAGGGCTGCT GTGCCCGGAT
CCCAGGCCCC TGGAGCTGGC ACTGCTCATC CTGGGCGTGG GGCTGCTGGA
CTTCTGTGGC CAGGTGTGCT TCACTCCACT GGAGGCCCTG CTCTCTGACC
TCTTCCGGGA CCCGGACCAC TGTCGCCAGG CCTACTCTGT CTATGCCTTC
ATGATCAGTC TTGGGGGCTG CCTGGGCTAC CTCCTGCCTG CCATTGACTG
GGACACCAGT GCCCTGGCCC CCTACCTGGG CACCCAGGAG GAGTGCCTCT
TTGGCCTGCT CACCCTCATC TTCCTCACCT GCGTAGCAGC CACACTGCTG
GTGGCTGAGG AGGCAGCGCT GGGCCCCACC GAGCCAGCAG AAGGGCTGTC
GGCCCCCTCC TTGTCGCCCC ACTGCTGTCC ATGCCGGGCC CGCTTGGCTT
TCCGGAACCT GGGCGCCCTG CTTCCCCGGC TGCACCAGCT GTGCTGCCGC
ATGCCCCGCA CCCTGCGCCG GCTCTTCGTG GCTGAGCTGT GCAGCTGGAT
GGCACTCATG ACCTTCACGC TGTTTTACAC GGATTTCGTG GGCGAGGGGC
TGTACCAGGG CGTGCCCAGA GCTGAGCCGG GCACCGAGGC CCGGAGACAC
TATGATGAAG GCGTTCGGAT GGGCAGCCTG GGGCTGTTCC TGCAGTGCGC
CATCTCCCTG GTCTTCTCTC TGGTCATGGA CCGGCTGGTG CAGCGATTCG

GCACTCGAGC AGTCTATTTG GCCAGTGTGG CAGCTTTCCC TGTGGCTGCC
GGTGCCACAT GCCTGTCCCA CAGTGTGGCC GTGGTGACAG CTTCAGCCGC
CCTCACCGGG TTCACCTTCT CAGCCCTGCA GATCCTGCCC TACACACTGG
CCTCCCTCTA CCACCGGGAG AAGCAGGTGT TCCTGCCCAA ATACCGAGGG
GACACTGGAG GTGCTAGCAG TGAGGACAGC CTGATGACCA GCTTCCTGCC
AGGCCCTAAG CCTGGAGCTC CCTTCCCTAA TGGACACGTG GGTGCTGGAG
GCAGTGGCCT GCTCCCACCT CCACCCGCGC TCTGCGGGGC CTCTGCCTGT
GAtGTCTCCG TACGTGTGGT GGTGGGTGAG CCCACCGAGG CCAGGGTGGT
TCCGGGCCGG GGCATCTGCC TGGACCTCGC CATCCTGGAT AGTGCCTTCC
TGCTGTCCCA GGTGGCCCCA TCCCTGTTTA TGGGCTCCAT TGTCCAGCTC
AGCCAGTCTG TCACTGCCTA TATGGTGTCT GCCGCAGGCC TGGGTCTGGT
CGCCATTTAC TTTGCTACAC AGGTAGTATT TGACAAGAGC GACTTGGCCA
AATACTCAGC Gggtggacac catcaccatc accattaa

Amino acid sequence (SEQ ID NO:9)

MSFLNFTAVL FAASSALAAP VNTTTEDETA QIPAEAVIGY SDLEGDFDVA
VLPFSNSTNN GLLFINTTIA SIAAKEEGVS LEKREAEAMV LGIGPVLGLV
CVPLLGSASD HWRGRYGRRR PFIWALSLGI LLSLFLIPRA GWLAGLLCPD
PRPLELALLI LGVGLLDFCG QVCFTPLEAL LSDLFRDPDH CRQAYSVYAF
MISLGGCLGY LLPAIDWDTS ALAPYLGTQE ECLFGLLTLI FLTCVAATLL
VAEEAALGPT EPAEGLSAPS LSPHCCPCRA RLAFRNLGAL LPRLHQLCCR
MPRTLRRLFV AELCSWMALM TFTLFYTDFV GEGLYQGVPR AEPGTEARRH
YDEGVRMGSL GLFLQCAISL VFSLVMDRLV QRFGTRAVYL ASVAAFPVAA
GATCLSHSVA VVTASAALTG FTFSALQILP YTLASLYHRE KQVFLPKYRG
DTGGASSEDS LMTSFLPGPK PGAPFPNGHV GAGGSGLLPP PPALCGASAC
DVSVRVVVGE PTEARVVPGR GICLDLAILD SAFLLSQVAP SLFMGSIVQL
SQSVTAYMVS AAGLGLVAIY FATQVVFDKS DLAKYSAGGH HHHHH

FIG.10: Saccharomyces cerevisiae (strain Y1790) P501S-His fermentation process

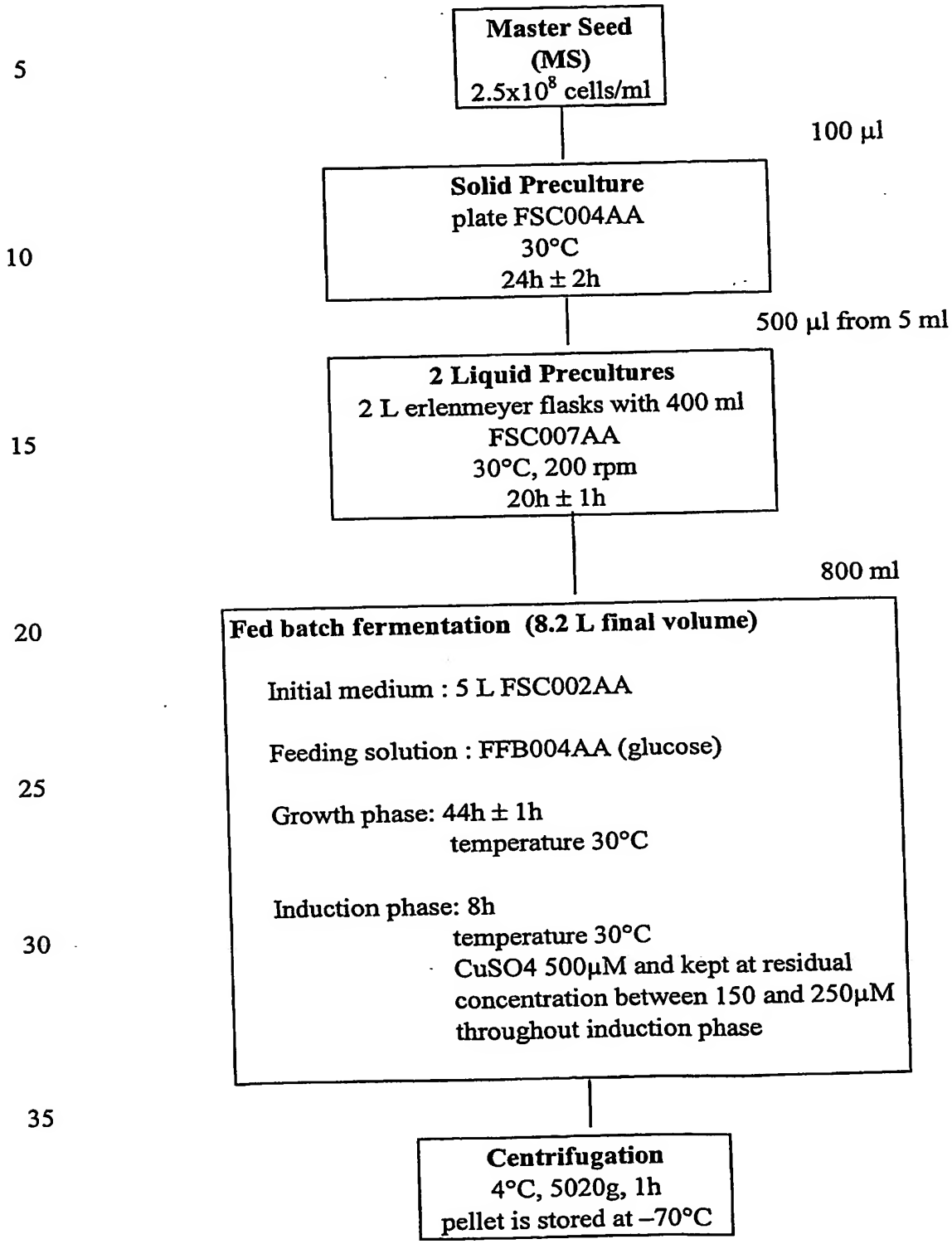

FIG.11: Real-time PCR analysis of P501S on Cynomolgus prostate and on a panel of rat tissues and cell lines.

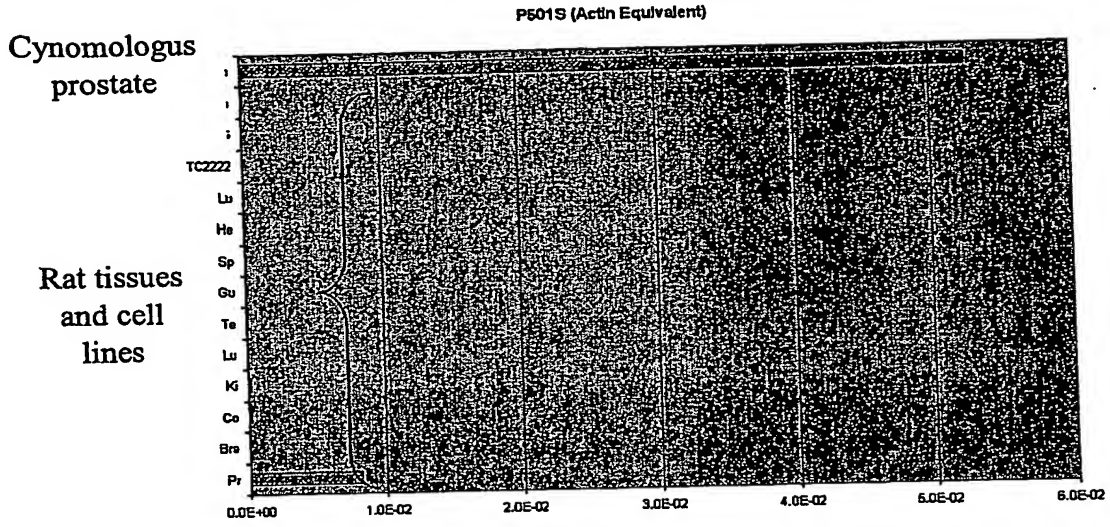

FIG. 12: amino acid sequence for mouse P501S (SEQ ID N°10)

MIQRLWASRLLRHRKAQLLLVNLLTFGLEVCLAAGITYVPPLLLEVGVEEKFMTMVL
GIGPVLGLVSVPLLGSASDQWRGRYGRRRPFIWALSLGVLLSLFLIPRAGWLAGLLY
PDTRPLELALLILGVGLLDFCGQVCFTPLEALLSDLFRDPDHCRQAFSVYAFMISLG
GCLGYLLPAIDWDTSVLAPYLGTQEECLFGLLTLIFLICMAATLFVTEEAVLGPPEP
AEGLLVSAVSRRCCPCHVGLAFRNLGTLFPRLQQLCCRMPRTLRRLFVAELCSWMAL
MTFTLFYTDFVGEGLYQGVPRAEPGTEARRHYDEGIRMGSLGLFLQCAISLVFSLVM
DRLVQKFGTRSVYLASVMTFPVAAAATCLSHSVVVVTASAALTGFTFSALQILPYTL
ASLYHREKQVFLPKYRGDAGGSSGEDSQTTSFLPGPKPGALFPNGHVGSGSSGILAP
PPALCGASACDVSMRVVVGEPPEARVVTGRGICLDLAILDSAFLLSQVAPSLFMGSI
VQLSHSVTAYMVSAAGLGLVAIYFATQVVFDKNDLAKYSV

FIG.13: nucleotide sequence encoding mouse P501S (SEQ ID N°11)

GAGATTTAAAAGGCGCCCGCTGGCGCGCGTTGGTGAMSCAGGBGTCGCCGAGCTCGC
ACGCGCCAGCCCCAGGTGACAGCCGCACGCCGGGCCAGGATCTGACCGACGAGATGT
GTCCCCATCAAGCAAGGCACTAGATGGTGACGTGTTTAGCGTGGGACGAGATGCTGA
ATTGGCACTAAAGGGCTGGCAGAAATGGGAACCTGGCTGCACCCTAGGAGGTTAGTG
CTAGTGAGGAGGAGAAGCCACGGCAGGGCTGACTCAAAGCAGCTGTGGAGTATGTGA
GTAGCCCCTGGAACCCTACCTGCCCTGTCCATCatgatccagaggctgtgggccagc
cgtctgctacggcaccggaaagctcagctcctgctggtcaacctgctcacctttggc
ctggaggtgtgcctggctgccggcattacctatgtgccacccctctgctggaagtc

ggggtggaggagaaattcatgaccatggtgttgggcattggcccagtgctaggcctg
gtttctgttccactcctaggctcagccagtgaccagtggcgtgggcgctatggccgc
cggagacccttatctgggctttgtccctgggtgtcctgctaagcctctttctcatc
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CAAAGAGAGGGTCTGCCTCATGGGTTCTCAGCCCTTAGAGGGCTGCAGAGCTGGCCT
CTCCAGGTCTTTGTCGCCTAAGTGGCTCTCTGCTGCCACCCTAAGGCAGTGAGGTGT
ATTGTTGCACAGATAGGAGCCAGAGCTTTCGGGGCTCTGGCTTCAGAGTCTGGCTGG
CCTACTGGCAGCCTCTCGCATG

FIG.14: Real-time PCR analysis of P501S on a panel of mouse tissues.